



Received: 6 Mar. 2014
Accepted: 2 Jul. 2014
Editor: R. Causse

Ichthyological note – Note ichtyologique

Melanic hyper-pigmentation in the genus *Lepidion* (Gadiformes: Moridae)

by

Rafael BAÑÓN* (1), Juan Carlos ARRONTE (2), Wolf ISBERT (2),
Germán COSCELLI (3) & Francisco SÁNCHEZ (2)

Résumé. – Hyper-pigmentation mélanique dans le genre *Lepidion* (Gadiformes: Moridae).

Deux cas d'hyperminéralisation mélanique sont décrits chez deux *Lepidion lepidion* provenant de deux régions différentes des eaux atlantiques espagnoles. Une recherche bibliographique a révélé la présence de cette anomalie chromatique chez deux autres spécimens de *Lepidion*, dont le premier a été signalé à la fin du 19^e siècle. Macroscopiquement, l'anomalie de couleur pigmentaire est caractérisée par la présence de nombreuses taches cutanées sombres et irrégulières sur la tête, le corps et les nageoires. Microscopiquement, la mélanose correspond à une prolifération hyperplasique des mélanophores dermiques. Bien que la cause de la prolifération hyperplasique des cellules pigmentées n'ait pu être déterminée, l'action possible d'agents étiologiques a été proposée. Ce travail constitue la première étude histopathologique d'un spécimen mélanique du genre *Lepidion*.

Keywords. – Moridae - *Lepidion* sp. - North-eastern Atlantic - Galicia Bank - Fish disease - Hyper-pigmentation - Melanosis.

Pigmentation and integumentary colours in fish are the result of a combination of coloured substances or biochromes, contained in various pigmented cells or chromatophores. There are three major chromatophore cell types: melanophores, xanthophores and iridophores (Fujii, 1993). Melanophores contain melanin, which gives a brown or black colouration. In teleosts, melanophores are commonly located in the dermis of the skin, forming a pigmented layer, but they can also appear in the epidermis and hypodermis (Beeching *et al.*, 2013). The pigmentation patterns are regulated by several intrinsic physiological conditions (Quigley and Parichy, 2002) and they are also influenced by numerous environmental stressors such as exposure to light and ultraviolet ray, temperature, osmolarity and pH of the water, mechanical pressure and nutrition (Greenwood *et al.*, 2012).

Many fish species may develop skin pigment abnormalities both in wild or farming conditions, mainly associated to melanophore disorders. Hyper-pigmentation is one of these disorders, characterized by the occurrence of focal or generalized spots, patches or bands of dark coloration (Groff, 2001; Simon *et al.*, 2009). It has been commonly observed associated with pathological conditions such as chronic inflammation, hyperplastic or neoplastic proliferation of melanophores (Roberts, 2012; Lévesque *et al.*, 2013).

Skin lesions, including hyper-pigmentation, have received considerable attention in the last few years, as they represent indicators of water pollution and/or otherwise stressed aquatic environments (Vethaak and Jol, 1996). The purpose of this study is to describe the occurrence of two specimens of *Lepidion lepidion* displaying external hyper-pigmentation. In one specimen, the hyper-pigmented skin was evaluated macroscopically and characterized by histopathological analysis.

MATERIALS AND METHODS

Two specimens of *L. lepidion* with skin hyperpigmentation were recorded in Spanish Atlantic waters (NE Atlantic). One specimen, measuring 345 mm total length (Fig. 1A), was caught by bottom trawl on 18 August 2010 on the Galician Bank (42°34'N; 11°57'W) at a depth of 1100 m during the research survey INDEMARES-BANGAL 0810. The specimen was frozen onboard, then fixed in 10% neutral buffered formalin. It was later transferred to 70% ethanol and deposited in the fish collection of the Instituto Español de Oceanografía in Santander (IEOST10052). The second specimen of about 320 mm (video-recorded specimen) was filmed alive in its natural habitat by the remotely operated vehicle (ROV) *Liropus 2000* on 4 May 2012 during a research survey (INDEMARES-AVILES 0412) in a cold-water coral reef in the La Gavira Canyon (43°55'N; 5°46'W) at a depth of 792 m (Fig. 2).

Macroscopic and microscopic examination

A detailed examination of fins and body for external parasites, malformations, amputations and any other morphological alterations of the captured specimen was carried out. A sample of skin and muscle (~2 cm²) with a distinct melanotic pigmentation was randomly selected and removed from the body to detect the presence of metazoan parasites. The muscle was separated from the skin and the tissue was sliced and squeezed between two glass plates and examined under a stereomicroscope with transmitted light. Likewise, samples of skin and underlying skeletal muscle, including hyper-pigmented and adjacent normal areas were removed for histopathological analysis. Fixed tissues were routinely processed and paraffin-embedded. Sections of 3-4 µm-thickness were cut and stained with haematoxylin and eosin (H-E), periodic acid-Schiff (PAS) and Fontana-Masson (FM). Alternatively, additional sections were bleached with 3% H₂O₂ for 24 h (in a humid chamber

(1) Servizo de Planificación, Dirección Xeral de Desenvolvemento Pesqueiro, Consellería do Mar e Medio Rural, Xunta de Galicia, Rua dos Irmandiños s/n, 15701 Santiago de Compostela, Spain.
(2) Instituto Español de Oceanografía, C.O. de Santander, Promontorio San Martín s/n, 39004 Santander, Spain. [jc.arronte@st.ieo.es] [wisbert@gmx.net] [fsanchez@st.ieo.es]
(3) Facultad Ciencias Veterinarias, Dpto. de Ciencias Clínicas Veterinarias, Campus Universitario, Pabellón 3, Estrada da Granxa s/n, 27002, Lugo, Spain. [gcoscelli@hotmail.com]

* Corresponding author [anoplogaster@yahoo.es]

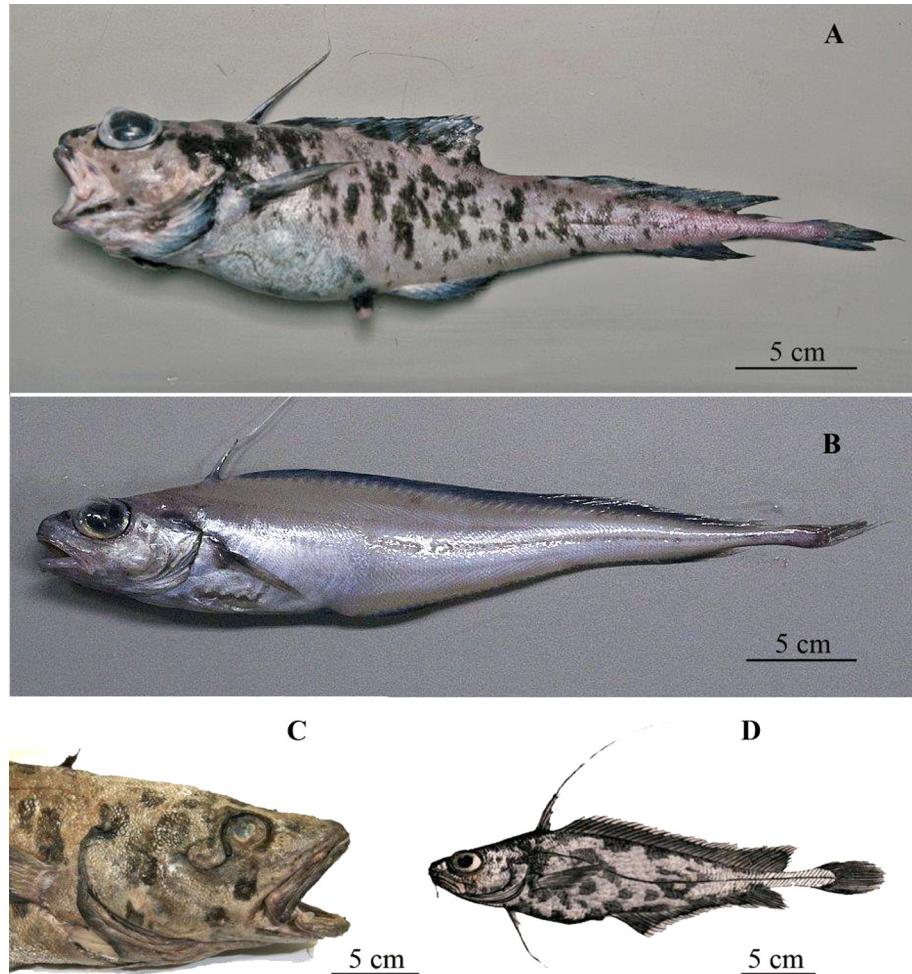


Figure 1. - Melanic and normal pigmentation in the genus *Lepidion*. A: Hyperpigmentation in *L. lepidion* IEOST10052; B: Specimen of *L. lepidion* showing a normal pigmentation; C: Detail of *L. guentheri* specimen reported in Bañón et al. (2010); D: *L. lepidion* in Moreau, 1881.

at room temperature) before H-E stainings. Skin samples of three specimens displaying normal pigmentation were used as a reference for normal histological structure.

RESULTS

Normal pigmentation

The typical pigmentation of *L. lepidion* is uniformly pale, varying from light brown to grey-pink, with the fin extremities lightly pigmented (Fig. 1B).

Altered skin pigmentation

Macroscopic examination

The captured and video-recorded specimens of *L. lepidion*, both exhibited darkened skin with evident hyper-pigmented areas, including the head, operculum and body surface. In the captured specimen, lesions were characterized by generalized, multifocal dark brown and black macules and spots with a smooth surface (Fig. 1A). Lesions were variable in shape and size, varying from well-demarcated spots to irregular and diffuse patches. Transverse sections of the body showed that hyper-pigmentation was restricted to the skin and was not found deep in the musculature. In addition, the captured fish exhibited a partial amputation on its dorsal profile,



Figure 2. - Specimen of *Lepidion lepidion* with advanced melanistic hyperpigmentation swimming over *Madrepora aculeata* and *Cerianthus* sp. in La Gaviera Canyon (Cantabrian Sea).

specifically in the middle of its second dorsal fin. The video-recorded specimen exhibited a similar skin colour pattern, but a higher proportion of skin surface was covered by hyper-pigmentation.

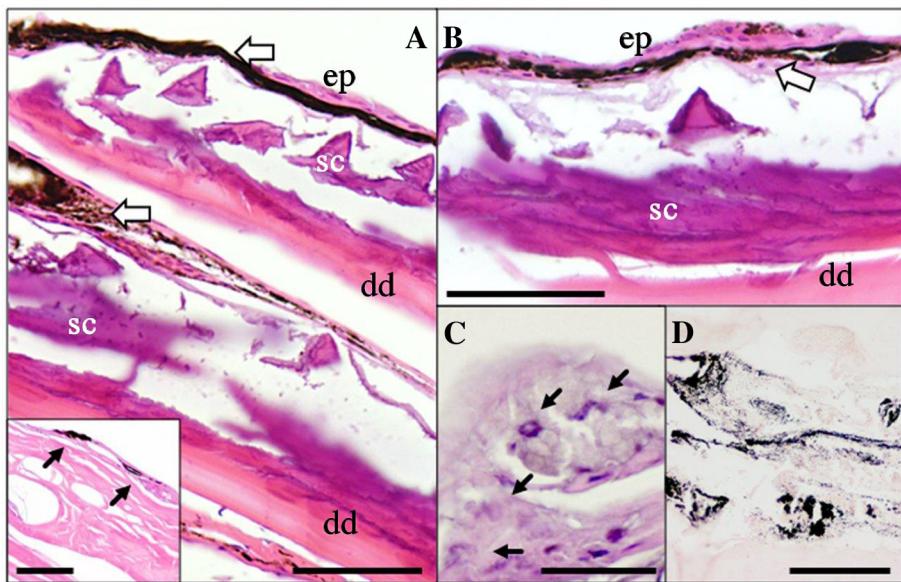


Figure 3 - Microphotographs of hypermelanized skin sections. A: Pigmented cell proliferation in the superficial dermis (stratum laxum) (arrows). H-E staining. Insert: distribution of melanophores (arrows) in normal pigmented skin. H-E staining. B: Hyperplasia of melanophores in superficial dermis forming a thick heavily pigmented layer beneath the epidermis (arrow). H-E staining. C: Foci of hyperplastic melanophores densely clustered. H-E staining. D: Positive argentaffin reaction of melanin granules. FM staining. dd: deep dermis; ep: epidermis; sc: scale. Scale bars: A, B = 50 µm; C = 20 µm; D = 100 µm.

Microscopic examination

The parasitological examination did not reveal any parasites either on the skin or in the muscular tissue. Histological examination of hyper-pigmented skin showed hyperplasia of dermal melanophores (Fig. 3A). Pigmented cells were largely distributed throughout the stratum laxum of the dermis, beneath the epidermis and above the scales, but not extending into the deep dermis. They formed a thick, continuous and compact pigmented row, parallel to the basement membrane (Fig. 3B). Demelanized samples showed melanophores with rounded to fusiform nuclei and indistinguishable cytoplasmic edges (Fig. 3C). Mitotic figures were not observed. Staining with the FM technique confirmed the presence of melanin granules in pigmented cells (Fig. 3D). There was no evidence of inflammatory or necrotic changes. No bacteria, fungi or parasites were observed in any of the analysed sections.

DISCUSSION

Following a recent revision of the genus, the Atlantic *L. eques* has been proposed as a junior synonym of the Mediterranean *L. lepidion* (Bañón et al., 2013). Therefore, *L. lepidion* is a deep-water species widely distributed in the North Atlantic Ocean and the Mediterranean Sea.

Macroscopically, the anomalous colouration pattern was quite similar in both specimens. A similar colouration pattern was previously reported in a specimen of the congeneric *L. guentheri* from the Bay of Biscay (Bañón et al., 2010) (Fig. 1C) and in a specimen of *L. lepidion* from the Mediterranean Sea (Moreau, 1881; Vinciguerra, 1883) (Fig. 1D). Thus, Moreau's original drawing (1881: 262) is the first documented case of melanosis in the genus *Lepidion* and one of the first, if not the first, among marine fishes.

Microscopically, the histopathological examination of tissues revealed that the skin melanosis corresponded with a severe hyperplasia of dermal melanophores, which resulted in the darkened skin. However, the aetiology of melanophore hyperplasia could not be established. Hyperplasia is characterized by an increase in organ size or tissue involved. It can be caused by an excessive and/or prolonged stimulation of hormones or growth factors on target cells, but also by inflammatory response to certain bacterial and viral

infections or physical agents, such as radiation or trauma (Cockrell and Cooper, 2002; Sweet et al., 2012; Lévesque et al., 2013). Melanophore hyperplasia seems to be a frequent cause of hyperpigmentation in teleost fishes and has been described previously in other fish species, such as *Sebastes* sp., *Pagellus acarne*, *Limanda limanda* and *Xiphophorus* sp. (Gimenez-Conti et al., 2001; Nogueira et al., 2013; Ramos et al., 2013).

The microscopic examination showed neither dermal inflammatory changes nor histological evidence of viral, bacterial, fungal or parasite infection. However, a preceding inflammatory process cannot be ruled out because the inductor stimuli could have been activated long before the specimen was examined. The lesion observed in the second dorsal fin of the captured fish may represent a sequel to a traumatic event, which may have been the initial stimulus that triggered the proliferation of melanophores. Although aetiology was not considered in the original publication, the specimen of *L. guentheri* with hyper-pigmentation reported by Bañón et al. (2010) also showed a sequel scar as a result of a traumatic injury in the suborbital area of the right eye (Fig. 1C). Unfortunately, the presence of wounds or injuries in both the Moreau's drawing and the video-recorded specimen of *L. lepidion* could not be confirmed. Chromatic alterations in fish species related to traumas, wounds and injuries have previously been reported in other fish species as *Carassius auratus* (Smith, 1931) and flatfishes (Norman, 1934; Dahlberg, 1970).

The four compiled cases confirm the presence of melanic hyperpigmentation within the genus *Lepidion*. However, considering the relatively small number of melanic specimens found to date, the prevalence of this condition in wild populations seems to be low. Nevertheless, specific attention to these anomalies is required in order to achieve a more comprehensive knowledge about the occurrence of melanic hyper-pigmentation in deep-water fish species.

Acknowledgements. – The research surveys INDEMARES-BANGAL 0810 and INDEMARES-AVILES 0412 were partially funded by the European Commission LIFE+ "Nature and Biodiversity" call, and included in the INDEMARES Project (07/NAT/E/000732). We are deeply grateful to Declan Quigley (Sea-Fisheries Protection Authority, Howth, Co Dublin,

Ireland) for his valuable comments on the manuscript. The Autonomous Government of Galicia (Xunta de Galicia) has collaborated in this project.

REFERENCES

BAÑÓN R., ARRONTE J.C., HEREDIA J. & PIS-MILLÁN J.A., 2010. - First record of a specimen of *Lepidion guentheri* (Giglioli, 1880) (Gadiformes: Moridae) with melanistic coloration. *J. Appl. Ichthyol.*, 26: 602-605.

BAÑÓN R., ARRONTE J.C., VÁZQUEZ-DORADO S., DEL RÍO J. & DE CARLOS A., 2013. - DNA barcoding of the genus *Lepidion* (Gadiformes: Moridae) with recognition of *Lepidion eques* as a junior synonym of *Lepidion lepidion*. *Mol. Ecol. Res.*, 13: 189-199.

BEECHING S.C., GLASS B.A. & REHOREK S.J., 2013. - Histology of melanic flank and opercular color pattern elements in the Firemouth Cichlid, *Thorichthys meeki*. *J. Morphol.*, 274(7): 743-749.

COCKERELL G.L. & COOPER B.J., 2002. - Disorders of cell growth and cancer biology In: Mechanisms of Disease: A Textbook of Comparative General Pathology (Slauson D.O. & Cooper B.J., eds), pp. 299-376. St. Louis, USA.

DAHLBERG M.D., 1970. - Frequencies of abnormalities in Georgia estuarine fishes. *Trans. Am. Fish. Soc.*, 99: 95-97.

FUJII R., 1993. - Cytophysiology of fish chromatophores. *Int. Rev. Cytol.*, 143: 191-255.

GIMENEZ-CONTI I., WOODHEAD A., HARSHBARGER J.C., KAZIANIS S., SETLOW R.B., NAIRN R.S. & WALTER R.B., 2001. - A proposed classification scheme for *Xiphophorus* melanomas based on histopathological analyses. *Mar. Biotechnol.*, 3(1): S100-S106.

GREENWOOD A.K., CECH J.N. & PEICHELA C.L., 2012. - Molecular and developmental contributions to divergent pigment patterns in marine and freshwater sticklebacks. *Evol. Dev.*, 14(4): 351-362.

GROFF J.M., 2001. - Cutaneous biology and diseases of fish. *Vet. Clin. North. Am. Exot. Anim. Pract.*, 4(2): 321-411.

LÉVESQUE M.P., KRAUSS J., KOEHLER C., BODEN C. & HARRIS M.P., 2013. - New tools for the identification of developmentally regulated enhancer regions in embryonic and adult zebrafish. *Zebrafish*, 10(1): 21-29.

MOREAU E., 1881. - Histoire naturelle des Poissons de la France. Vol. 3, 698 p. Paris: Masson.

NOGUERA P.A., FEIST S.W., BATEMAN K.S., LANG T., GRÜTJEN F. & BRUNO D.W., 2013. - Hyperpigmentation in North Sea dab *Limanda limanda*. II. Macroscopic and microscopic characteristics and pathogen screening. *Dis. Aquat. Org.*, 103: 25-34.

NORMAN J.R., 1934. - A Systematic Monograph of Flatfishes (Heterosomata). Vol. I: Psettodidae, Bothidae, Pleuronectidae. 463 p. London: British Museum (Natural History).

QUIGLEY I.K. & PARICHY D.M., 2002. - Pigment pattern formation in zebrafish: a model for developmental genetics and the evolution of form. *Microsc. Res. Tech.*, 58: 442-455.

RAMOS P., VICTOR P. & BRANCO S., 2013. - Spontaneous melanotic lesions in axillary seabream, *Pagellus acarne* (Risso). *J. Fish Dis.*, 36(9): 769-777.

ROBERTS R.J., 2012. - Fish Pathology. 4th edit., 590 p. Wiley-Blackwell.

SIMON T., JOYEUX J.C. & MACIEIRA R.M., 2009. - First record of partial melanism in the coney *Cephalopholis fulva* (Perciformes: Epinephelidae). *Braz. J. Oceanogr.*, 57(2): 145-147.

SMITH G.M., 1931. - The occurrence of melanophores in certain experimental wounds of the goldfish (*Carassius auratus*). *Biol. Bull.*, 61(1): 73-84.

SWEET M., KIRKHAM N., BENDALL M., CURREY L., BYTHELL J. & HEUPEL M., 2012. - Evidence of melanoma in wild marine fish populations. *PLoS ONE*, 7: e41989.

VETHAAK A.D. & JOL J.C., 1996. - Diseases of flounder *Platichthys flesus* in Dutch coastal and estuarine waters, with particular reference to environmental stress factors. I. Epizootiology of gross lesions. *Dis. Aquat. Org.*, 26: 81-97.

VINCIGUERRA D., 1883. - Risultati Ittiologici delle crociere del "violante". *Ann. Mus. Civ. Stor. Nat. Genova*, 18: 465-590.